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Re: **Claims for Divisional application** for
United States Patent Application No. 09/580,515 (09010-029005)
For: RECOMBINANT BACTERIAL PHYTASES AND USES THEREOF
Applicant: Jay M. Short et al.
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1. A **vector** comprising a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, its complementary sequence, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, its complementary sequence, or a subsequence thereof, (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, its complementary sequence, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2.

2. The vector of claim 1, wherein the vector comprises a plasmid, a viral vector, a mammalian expression vector, a phage, a plasmid, a phagemid, a cosmid, a fosmid, a bacterial artificial chromosome, a P1 based artificial chromosomes, a yeast plasmid, a yeast artificial chromosome, a viral particle, a derivative of SV40 or a baculovirus.

3. The vector of claim 1, wherein the nucleic acid further comprises introns or non-coding sequence 5' or 3' of the coding sequence.

4. The vector of claim 1, wherein the nucleic acid further comprises and is operably linked to a regulatory sequence.

5. The vector of claim 4, wherein the regulatory sequence comprises a promoter or a transcription control sequence.

6. The vector of claim 5, wherein the promoter or the transcription control sequence is operable in a plant cell, a plant part or a plant.

7. The vector of claim 5, wherein the promoter a tissue-specific promoter or a constitutive promoter.

8. The vector of claim 7, wherein the promoter is specific in a plant cell, a plant part or a plant.

9. The vector of claim 1, wherein the nucleic acid encodes a polypeptide having a phytase activity.

10. The vector of claim 9, wherein the polypeptide having a phytase activity comprises a phytase enzyme.

11. The vector of claim 1, wherein the allelic variant has a substitution, deletion or addition of one or more nucleotides.

12. The vector of claim 11, wherein the allelic variant has a substitution, deletion or addition of one or more nucleotides which does not substantially alter the function of an encoded phytase enzyme.

13. The vector of claim 1, wherein the nucleic acid further comprises a polynucleotide encoding a signal peptide, a tag or a fluorescent protein.

14. The vector of claim 13, wherein the signal peptide is a PR protein PR-S signal peptide from tobacco.

15. **A recombinant expression system** comprising a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, its complementary sequence, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, its complementary sequence, or a subsequence thereof, (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, its complementary sequence, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2, wherein the nucleic acid is operably linked to a promoter.

16. The recombinant expression system of claim 15, further comprising an *in vitro* transcription system.

17. The recombinant expression system of claim 15, further comprising an *in vivo* transcription system.

18. The recombinant expression system of claim 17, wherein the *in vivo* transcription system comprises a cell.

19. The recombinant expression system of claim 18, wherein the cell is a bacterial cell, a mammalian cell, a yeast cell, an insect cell, a plant cell or seed.

20. **A transformed cell or seed** comprising a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, its complementary sequence, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, its complementary sequence, or a subsequence thereof, (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, its complementary sequence, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2.

21. The transformed cell or seed of claim 20, wherein the cell is a prokaryotic cell or a eukaryotic cell.

22. The transformed cell or seed of claim 21, wherein the cell is a bacterial cell, a mammalian cell, a yeast cell, an insect cell, a plant cell or a plant seed.

23. The transformed cell or seed of claim 22, wherein the plant cell is a monocot or a dicot.

24. The transformed cell or seed of claim 22, wherein the plant is a cauliflower, an artichoke, an apple, a banana, a berry, a currant, a cherry, a cucumber, a grape, a lemon, a melon, a nut, a citrus, an orange, a peach, a pear, a plum, a strawberry, a tomato, a leaf, an alfalfa, a cabbage, an endive, a leek, a lettuce, a spinach, a tobacco, a root, an arrowroot, a beet, a carrot, a cassava, a turnip, a *Brassica*, a radish, a yam, a sweet potato, a seed, a bean, a pea, a soybean, a wheat, a barley, a corn, a rice, a rapeseed, a millet, a sunflower, an oat, a tuber, a kohlrabi or a potato.

25. The transformed cell or seed of claim 23, wherein the dicot is a tobacco, a potato, a tomato, a *Petunia* or a *Brassica* plant.

26. The transformed cell or seed of claim 21, wherein the eukaryotic cell is a mammalian cell or a yeast cell.

27. **A feed or food or feed or food additive** comprising a transformed cell or seed as set forth in claim 20 or a vector as set forth in claim 1.

28. **A dietary aid** comprising a transformed cell or seed as set forth in claim 20 or a vector as set forth in claim 1.

29. **A non-biodegradable material** comprising a transformed cell or seed as set forth in claim 20 or a vector as set forth in claim 1.

30. The non-biodegradable material of claim 29, wherein the non-biodegradable material comprises a thermoplastic, an acrylic, a modacrylic, a polyamide, a polycarbonate, a polyester, a polyethylene, a polypropylene, a polystyrene, a polysulfone, a polyethersulfone, a polyvinylidene fluoride, an elastomer, a polyamide, a polyester, a polyethylene, a polypropylene, a polystyrene, a polyurethane, a polyvinyl alcohol or a silicone.

31. **A biodegradable material** comprising a transformed cell or seed as set forth in claim 20 or a vector as set forth in claim 1.

32. The biodegradable material of claim 31, wherein the biodegradable material comprises a poly(lactide), a poly(glycolide), a poly(lactic acid), a poly(glycolic acid), a polyanhydride, a polyorthoester, a polyetherester, a polycaprolactone, a polyesteramide, a polycarbonate, a polycyanoacrylate, a polyurethane or a polyacrylate.

33. **A transgenic plant, plant organ or plant part** comprising a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, its complementary sequence, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, its complementary sequence, or a subsequence thereof, or (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, its complementary sequence, or a subsequence thereof.

34. The transgenic plant, plant organ or plant part of claim 33, wherein the plant is a monocot or a dicot.

35. The transgenic plant, plant organ or plant part of claim 33, wherein the plant part comprises seeds, fruits, flowers, leaves, stems, roots, hypocotyls or cotyls.

36. **A feed or food or feed or food additive** comprising a transgenic plant, plant organ or plant part as set forth in claim 33.

37. **A dietary aid** comprising a transgenic plant, plant organ or plant part as set forth in claim 33.

38. **A non-biodegradable material** comprising a transgenic plant, plant organ or plant part as set forth in claim 33.

39. The non-biodegradable material of claim 38 wherein the non-biodegradable material comprises a thermoplastic, an acrylic, a modacrylic, a polyamide, a polycarbonate, a polyester, a polyethylene, a polypropylene, a polystyrene, a polysulfone, a polyethersulfone, a polyvinylidene fluoride, an elastomer, a polyamide, a polyester, a polyethylene, a polypropylene, a polystyrene, a polyurethane, a polyvinyl alcohol or a silicone.

40. **A biodegradable material** comprising a transgenic plant, plant organ or plant part as set forth in claim 33.

41. The biodegradable material of claim 40, wherein the biodegradable material comprises a poly(lactide), a poly(glycolide), a poly(lactic acid), a poly(glycolic acid), a polyanhydride, a polyorthoester, a polyetherester, a polycaprolactone, a polyesteramide, a polycarbonate, a polycyanoacrylate, a polyurethane or a polyacrylate.

42. **A non-human transgenic animal** comprising a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, its complementary sequence, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be

U, its complementary sequence, or a subsequence thereof, or (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, its complementary sequence, or a subsequence thereof.

43. The non-human transgenic animal of claim 42, wherein the animal is a non-ruminant animal.

44. The non-human transgenic animal of claim 42, wherein the animal is a monogastric animal.

45. **A method of treating a human or an animal** able to benefit from digestive enhancement by the activity of an exogenous phytase enzyme comprising administering to the human or animal an amount of exogenous phytase enzyme effective to provide a phytase activity in the human or animal digestive tract.

46. The method of claim 45, wherein the exogenous phytase enzyme is encoded by a nucleic acid comprising (i) a sequence as set forth in SEQ ID NO:1, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, or a subsequence thereof, (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2.

47. The method of claim 45, wherein the exogenous phytase enzyme further comprises a magnetic carrier.

48. The method of claim 45, wherein the exogenous phytase enzyme further comprises a biodegradable material or a non-biodegradable material.

49. The method of claim 45, wherein the exogenous phytase enzyme further comprises a porous hollow particle.

50. The method of claim 45, wherein the exogenous phytase enzyme further comprises a liposome.

51. The method of claim 45, wherein the exogenous phytase enzyme further comprises a dough or a food medium.

52. **A method of treating an animal** able to benefit from digestive enhancement by the activity of an exogenous phytase enzyme comprising administering to the animal an amount of plant seed, plant cell, plant part or transgenic plant, effective to provide a phytase activity in the animal's digestive tract, wherein the plant, plant seed, plant cell or plant part comprises an expression system comprising a nucleic acid encoding a phytase enzyme and an expressed phytase enzyme.

53. The method of claim 52, wherein the nucleic acid comprises (i) a sequence as set forth in SEQ ID NO:1, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, or a subsequence thereof, (iii) a nucleic acid encoding a polypeptide comprising a sequence as set forth in SEQ ID NO:2, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2.

54. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant is administered in the form of a dietary aid, a food or a feed.

55. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant is administered in the form of a liquid or a solid.

56. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant is administered to a mono-gastric animal.

57. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant is administered to an animal before, after, or simultaneously with a meal.

58. The method of claim 52, wherein an effective amount of the exogenous phytase enzyme in the dietary aid, food or feed is from about 10 to 20,000, from about 10 to 15,000, from about 10 to 10,000, from about 100 to 5,000, or from about 100 to about 2,000 FYT/kg.

59. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant further comprises a biodegradable material or a non-biodegradable material.

60. The method of claim 52, wherein an effective amount of the amount of plant seed, plant cell, plant part or transgenic plant is administered as a top dressing, by mixing into an animal feed or food, by oral dosage, by injection, by transdermal means or in combination with other edible compounds or liquids.

61. The method of claim 52, wherein the plant seed, plant cell, plant part or transgenic plant is administered in a form comprising a non-toxic pharmaceutically acceptable edible carrier.

62. The method of claim 61, wherein the edible carrier is a solid or a liquid.

63. The method of claim 61, wherein the edible carrier comprises a corn starch, a lactose, a sucrose, a soy flake, a peanut oil, an olive oil, a sesame oil or a propylene glycol.

64. The method of claim 61, wherein the edible carrier comprises a solid carrier in the form of a tablet, a capsule, a powder, a troche, a lozenge, a top dressing or a micro-dispersable form.

65. The method of claim 61, wherein the edible carrier comprises a liquid carrier in the form of a soft gelatin capsule, a syrup, a liquid suspension, an emulsion or a solution.

66. The method of claim 52, wherein an effective amount of the exogenous phytase enzyme in the dietary aid, food or feed is administered in a dosage form.

67. The method of claim 52, wherein an effective amount of the plant seed, plant cell, plant part or transgenic plant is administered with an adjuvant, a preservative, a stabilizer, a wetting agent, an emulsifying agent or a solution promoter.

68. The method of claim 52, wherein an effective amount of the plant seed, plant cell, plant part or transgenic plant is administered as a fast-release composition or a slow-release composition.

69. The method of claim 52, wherein the animal is a mammal.

70. The method of claim 69, wherein the animal is a human.

71. The method of claim 52, wherein the animal is a fowl or a fish.

72. The method of claim 52, wherein the animal is a pig, a cattle, a sheep, a goat, a laboratory rodent, a rat, a mouse, a hamster, a gerbil, a fur-bearing animal, a mink, a fox, a zoo animal, a monkey, an ape, a domestic mammal, a cat, a dog, an avian specie, a chicken, a turkey, a duck, a goose, a pheasant, an emu, an ostrich, a loon, a kiwi, a dove, a parrot, a cockatiel, a cockatoo, a canary, a penguin, a flamingo, a quail, a farmed fish, a trout, a tropical fish, a goldfish, a carp, a catfish, a salmon, a shark, a ray, a flounder, a sole, a tilapia, a medaka, a guppy, a molly, a platyfish, a swordtail, a zebrafish or a loach.

73. **A method of generating a nucleic acid encoding a phytase variant** comprising (i) providing a nucleic acid as set forth in claim 1; and, (ii) modifying one or more nucleotides in the nucleic acid to another nucleotide, deleting one or more nucleotides in the nucleic acid, or adding one or more nucleotides to the nucleic acid, thereby generating a nucleic acid encoding a phytase variant.

74. The method of claim 73, further comprising expressing the variant nucleic acid, thereby generating a phytase variant.

75. The method of claim 73, wherein the nucleic acid of step (i) comprises at least 30 consecutive nucleotides of a nucleic acid as set forth in claim 1.

76. The method of claim 73, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, gene site saturated

mutagenesis (GSSM) and any combination thereof.

77. The method of claim 73, wherein the modifications are introduced by error-prone PCR.

78. The method of claim 73, wherein the modifications are introduced by shuffling.

79. The method of claim 73, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.

80. The method of claim 73, wherein the modifications are introduced by assembly PCR.

81. The method of claim 73, wherein the modifications are introduced by sexual PCR mutagenesis.

82. The method of claim 73, wherein the modifications are introduced by *in vivo* mutagenesis.

83. The method of claim 73, wherein the modifications are introduced by cassette mutagenesis.

84. The method of claim 73, wherein the modifications are introduced by recursive ensemble mutagenesis.

85. The method of claim 73, wherein the modifications are introduced by exponential ensemble mutagenesis.

86. The method of claim 73, wherein the modifications are introduced by site-specific mutagenesis.

87. **An isolated or recombinant nucleic acid** generated by the method of claim 73.

88. **A transformed cell or seed or a transgenic plant or plant part** comprising an isolated or recombinant nucleic acid generated by the method of claim 73.

89. **A transgenic non-human animal** comprising an isolated or recombinant nucleic acid generated by the method of claim 73.

90. **A food, a feed or a dietary supplement** comprising the transformed cell or seed or a transgenic plant or plant part of claim 88.

91. A method of introducing a phytase activity into a plant, plant part or plant cell comprising introducing a nucleic acid into the plant, plant part or plant cell by transformation of protoplasts or introducing a vector into the plant, plant part or plant cell, wherein the nucleic acid comprises (i) a sequence as set forth in SEQ ID NO:1, or a subsequence thereof (ii) a sequence as set forth in SEQ ID NO:1, wherein T can also be U, or a subsequence thereof, (iii) a sequence encoding a polypeptide having a sequence as set forth in SEQ ID NO:2, or a subsequence thereof, or (iv) an allelic variant of a nucleic acid encoding a phytase enzyme having a sequence as set forth in SEQ ID NO:2; wherein the nucleic acid encodes a polypeptide having a phytase activity.

92. The method of claim 91, wherein the nucleic acid is introduced into the plant, plant part or plant cell by a calcium/ polyethylene glycol method, electroporation, microinjection or particle bombardment.

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Page 14 of 14

93. The method of claim 91, wherein the vector comprises a viral vector, a bacterial vector or a vector from the genus *Agrobacterium*.

94. The method of claim 91, wherein the viral vector comprises a Cauliflower Mosaic Virus (CaMV).